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REVIEW

ARE ADIPOCYTES AND ROS VILLAINS, OR ARE THEY PROTAGONISTS IN THE DRAMA OF LIFE? THE MURBURN PERSPECTIVE

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Abstract

Several questions remain unanswered regarding the roles and interactive dynamics of reactive oxygen species (ROS) and lipids/adipocytes. ROS have been conventionally associated with deleterious effects in biological systems, especially correlated with metabolic disorders exemplified by diabetes, obesity, cancer, atherogenesis, etc. Adipocytes, originally perceived as mere lipid-storing cells, were also associated with such metabolic disorders. In later times, adipose tissue was shown to have several favorable metabolic and physiological functions. Similarly, ROS were also acknowledged with favorable roles in cellular signaling. Very recently, ROS have been shown to be indispensable protagonists of key life-sustaining routines such as oxidative phosphorylation, thermogenesis and xenobiotics' metabolism. In the light of these developments, herein, we attempt to address why different research groups derive data that project/infer contrasting correlations of ROS and lipids with good health. We advocate that rather than seeing ROS and fat deposition as deleterious to health, the modality of their generation, loci of presence and the relative amounts/distribution in milieu are the crucial factors that determine their interactions/roles (and thereby, the resulting physiology!) in miscellaneous microenvironments.

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Introduction

For decades, ROS (reactive oxygen species) have been seen as toxic and wasteful byproducts of biological processes like oxidative phosphorylation and xenobiotic metabolism. The research for anti-oxidants (molecules that scavenged ROS) gained momentum due to the correlation of ROS in cancer progression, ageing, apoptosis and oxidative damage to genes and proteins (1-2). Similarly, fatty tissue and free-lipid deposition were seen to have primarily negative connotations towards maintenance of good health (3). In recent times, the perceptions in the pertinent field of ROS-lipid interactions have undergone significant changes and this write-up aims to capture the salient aspects of the interactive dynamics of ROS and lipids/adipocytes.

Various ROS and their well-studied roles

ROS (reactive oxygen species), constitutes a set of "atom-molecule-ion-radical" entities derived from the normal triplet molecular dioxygen, which is the abundant species present in atmosphere. This common oxygen species, $^3O_2$, is

thus represented because the presence of two unpaired electrons in the bonded structure gives a "triplet spin multiplicity" spectroscopic signature when a magnetic field is applied. Due to "spin rules", the triplet oxygen does not pose high reactivity with most biomolecules. Table 1 provides the details of oxygen and its derivatives that form the simplest classes of ROS. Besides the ones listed below, ozone (O_3, or trioxygen, a neutral triatomic molecule) and various other species incorporating nitrogen (e.g. nitric oxide, peroxynitrite), halogen (e.g. hypochlorous acid, chlorite, etc.), carbon (of the nature- RO*, ROO*, etc.) atoms, are also examples of more diverse types of ROS encountered in biological systems.

Of all the ROS species, the superoxide ion radical is the one most abundantly found in physiological pH and can also exist as hydroperoxyl radical which can, more easily, percolate through the phospholipid bilayer. Hydroxyl radical is a very highly reactive species that can damage nucleic acids, peptides and lipids severely. Hydrogen peroxide is relatively less reactive and formed mostly from the reaction of superoxide with the enzyme superoxide dismutase. Peroxide can cause damage to DNA by producing the hydroxyl radical. Singlet oxygen ($^1O_2$) is deemed to be extremely toxic and can cause DNA and tissue level damage. It is mostly produced during neutrophil activation. Ozone (O_3) is produced during inflammatory process *in vivo* and can be a strong oxidizing agent forming reaction intermediates with biological molecules that may lead to aberrations. Such pathophysiological interactions of ROS with biological molecules have been well documented in scientific literature (4-11). In the last couple of decades, the roles of ROS as molecular signals/messengers have been reported, explored and some cases like NO have been well-established (12-17).

Types of adipocytes and their perceived functions

Adipocytes are cells that store excess energy (lipid reserves) in the form of triglycerides and tissues incorporating such cells also provide mechanical support and insulation to organisms. Recently, adipose tissue is also considered a major endocrine and paracrine organ of the human organism. A typical demarcation of adipose tissue based on its coloration divides it into 2 types: brown adipose tissue (BAT) and white adipose tissue

Table 1. Electronic distribution and naming conventions of select oxygen-centered species

Oxygen species	Notation	Stature (charge) & relevant pK_a (if any)	Electronic orbitals						
Triplet Oxygen	$^3O_2$	Diatomic diradical molecule (0). (Normal dioxygen)	↑↓	↑↓	↑↓	↑↓	↑↓	↑	↑
Oxygen	O	Atom (0); transient and very reactive	↑↓	↑↓	↑↓				
Singlet Oxygen	$^1O_2$	Diatomic molecule (0) (Excited dioxygen; more reactive to organics than singlet oxygen)	↑↓	↑↓	↑↓	↑↓	↑↓	↑↓	
Superoxide	O_2^{*-}	Diatomic radical ion (-); becomes triatomic perhydroxyl/hydroperoxyl uncharged radical on protonation at pH 4.8. (Protonated species more reactive to organics than superoxide ion.)	↑↓	↑↓	↑↓	↑↓	↑↓	↑↓	↑
Peroxide	H_2O_2	Tetra-atomic molecule (0); somewhat reactive, becomes triatomic hydroperoxide ion on deprotonation at 11.7.	↑↓	↑↓	↑↓	↑↓	↑↓	↑↓	↑↓
Hydroxyl	OH^*	Di(hetero)atomic uncharged radical (0), one of the most reactive species known, 11.5. Deprotonated species (O^{*-}) less reactive.	↑↓	↑↓	↑↓	↑			
Hydroxide	OH^-	Di(hetero)atomic ion (-); not very reactive, becomes triatomic water molecule on protonation.	↑↓	↑↓	↑↓	↑↓			
Oxide	O^{2-}	Monoatomic ion (--)	↑↓	↑↓	↑↓	↑↓			
Water	H_2O	Triatomic molecule (0), 14.	↑↓	↑↓	↑↓	↑↓			

(WAT). The multilocular BAT contains lower levels of lipids and more number of mitochondria when compared to WAT (18). Brown adipose tissue was earlier perceived as specific to infants and rodents as a site for non-shivering thermogenesis but recent finds have revealed its presence in adult humans as well. The mechanism of thermogenesis in BAT was essentially understood to be owing to two phenomena: lipolysis and dissipation of transmembrane potential formed by proton pumps in mitochondrial membrane (19). Some WAT undergo browning and are called "inducible, beige, or brite" adipose tissue (20). Figure 1 shows the schematic differentiation of the three types of adipose cells/tissues. However, recent studies also show that adipose tissue is not homogenous and varies in their metabolic profiles by depot-specificity and location (21). At present, it is not exactly clear how the various genetic and hormonal controls achieve the switching of different types of adipose tissues.

An update on the origin and relevance of ROS in biological systems

Several external agents are known to act as sources that lead to generation of ROS, as shown in the top panel of Figure 2. The parsing of these agents is into three types – physical, chemical and foodstuff. External physical agents like various high-energy photons (of X-rays, cosmic rays, gamma rays, UV-light, etc.) and ionizing radiations (like alpha and beta rays) could impart sufficient energy for molecular breakage at various sites, leading to the generation of various radicals and ROS within (22-23). Chemicals like natural alkaloids, xenobiotics like halogenated organics, heavy metals and their salts, etc. are known ROS generators. Besides, foodstuff as exemplified by processed meat, used oil, alcoholic beverages, etc. are also known to lead to ROS generation. The function of ROS within the organism has been viewed under two lights- primarily pathological implications (immune responses, phagocytosis, and several diseases/disorders) and secondarily for the purpose of molecular signaling (like nitric oxide, superoxide, etc.). The internal sites/organelles where ROS generation is known to be high are- mitochondria, endoplasmic or sarcoplasmic reticulum, and peroxisomes. This is the classical way of seeing ROS dynamics with respect to its interaction with various agents.

Our group's research works have dwelled on the utility of ROS as an electron transfer and group transfer agent in routine metabolism. We have shown that ROS and diffusible species are responsible for electron/moiety transfers seen in heme/flavin enzymes mediated reactions (24-35), which explain the metabolism of xenobiotics in hepatocyte endoplasmic reticulum

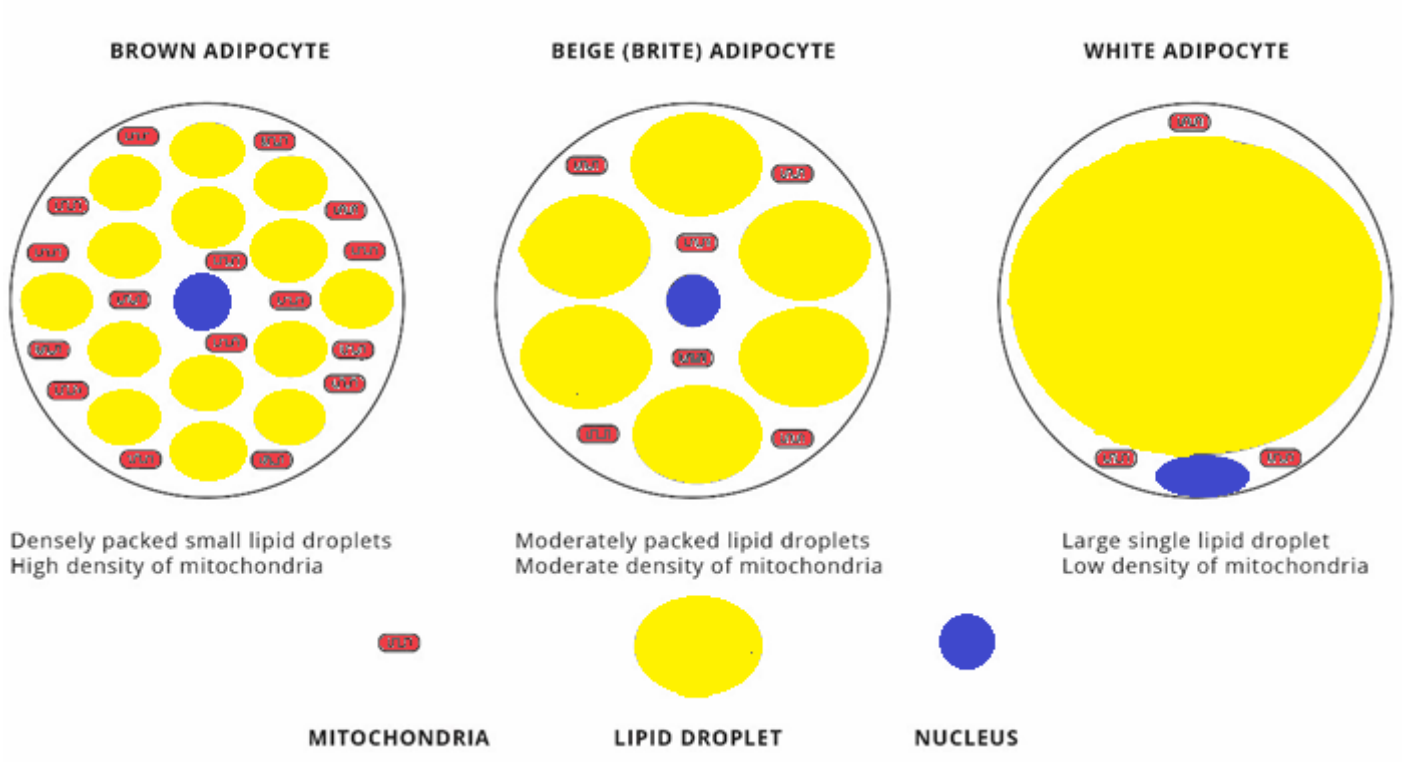

Figure 1. Schematic rendition of the different types of adipocytes

(microsomes) (36-38), oxidative phosphorylation in mitochondria (39-41), thermogenesis mediated by uncoupling proteins in BAT (41-42), and maverick physiological dose responses (35, 43). This new metabolic/physiological paradigm that invokes reactions mediated via diffusible reactive species was recently called *murburn concept* (34-41, 43). Therefore, it is now definitive that production of ROS in cellular systems cannot be seen as a pathological or cellular signaling process alone. The generation and utilization of ROS should now be seen as an obligatory "catalytic outcome" that resulted due to evolutionary pressures. This is because cells evolved to capitalize on the fast reactions mediated by oxygen centered radicals and the high potentials of these radicals could activate even non-activated carbons, enabling facile metabolism. The predominantly hydrophobic organic molecules in the lipid cellular membranes would have high adsorption for the organic molecules. Retention of these molecules would progressively destroy cell structure and function. So, the generation of ROS enabled the attack on these xenobiotics or alien molecules, thereby rendering them polar (as a hydroxyl group is introduced) and that would in turn be subjected to a "cleansing out" by water, the solvent of life. If ROS were to be bad, we cannot explain why millions of years of evolution could not bring about the structural mandates so that the "deleterious and chaotic" ROS were not produced by the proteins/organelles of prokaryotes and eukaryotes.

Therefore, a more holistic functional classification for ROS dynamics is presented within the bottom panel of Figure 2. The new perspective is captured by three "actions"- introduction, induction and production. ROS can be directly introduced into the living system (via smoke or tissue burning) or indirectly induced within the organism by an exposure to physical agents (like high-energy or ionizing radiations). Internal (*in situ* or *in*

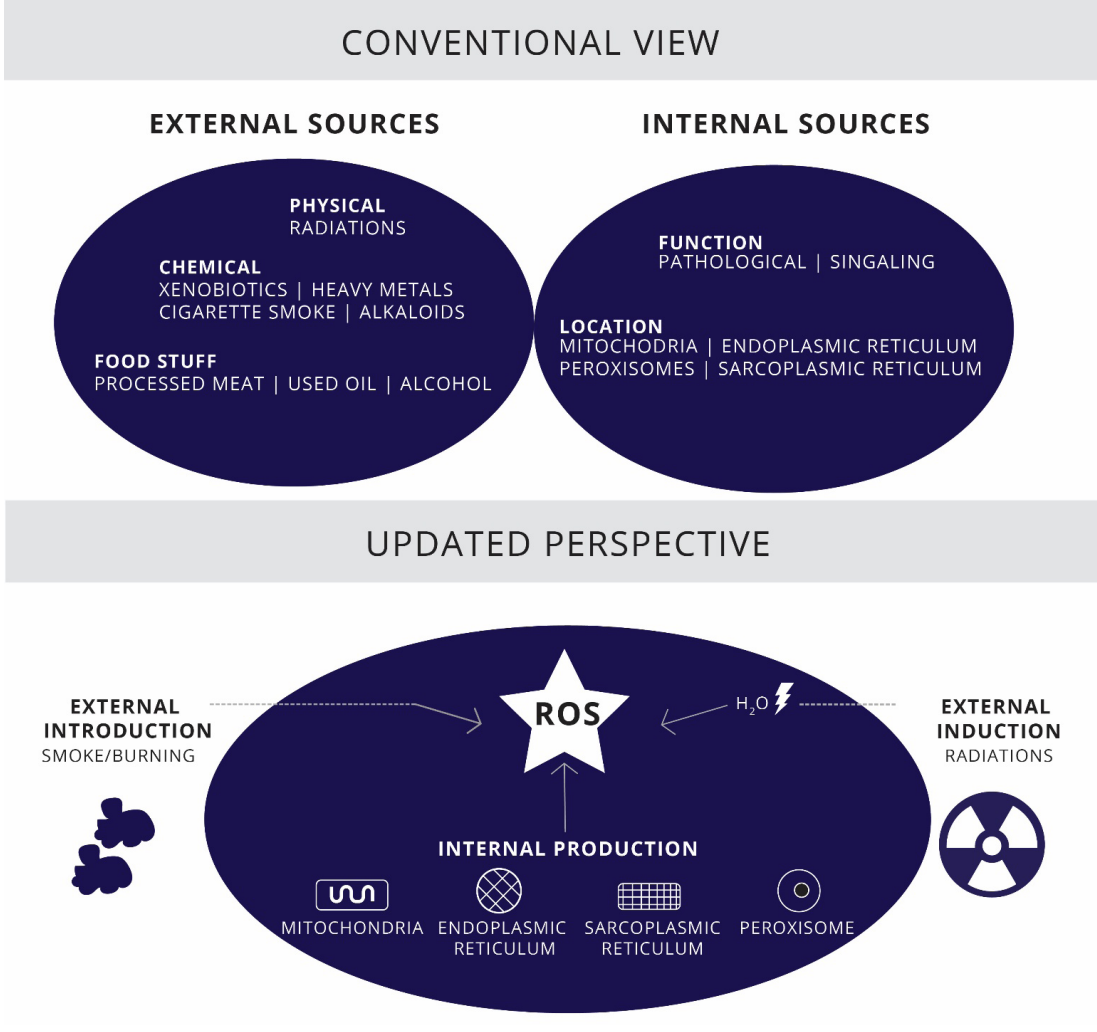

Figure 2. ROS dynamics within cells: conventional and updated perceptions

vivo) metabolism of several molecules occurs with the obligatory production and utilization of ROS at mitochondria, endoplasmic/sarcoplasmic reticulum, peroxisomes, etc. Therefore, ROS production within cells is no more to be seen as a pathological attribute (wasteful or toxic side-product). However, this is not to say that ROS is always beneficial. This metabolic facet is both physiological and at times, could end up being pathological. Just like any radical with high reaction potential, ROS is also capable of grave damage to living systems. The paradigm change of ROS from being a villain (pathological effects), to the side-kick (cellular messenger) and finally to a benevolent cum indispensable protagonist (catalytic agent) is evident with the analysis of scientific literature through the past decades (13, 40-41, 43-46).

The perception of functional roles played by free-lipids and lipid-laden cells/tissues

Just like the change in perceptions on ROS, the case of adipose tissue has also undergone a radical change over the decades. Adipose tissue was also seen under negative light with increasing incidence of obesity and associated metabolic pathology (18, 47). However, recently, adipocytes have been acknowledged for their role in nutrient homeostasis, energy balance and release of adipokines, endocrine signaling molecules and protein factors (18, 48- 50). Also, it is known that Eskimos eat animal blubber in huge amounts, and they still have a long, active and healthy life. While low density lipoproteins are deemed bad, high density lipoproteins are good. Therefore, lipids and their storage depots (adipocytes), per se, cannot be bad for health. Lipids (and not protein-capsids or glycan-walls!) evolved as the fundamental biological membrane choice because of its inherent fluidity and electron-rich nature that could form organized structures and embed proteins. The obligatory requirement and favorable roles of lipids in life is endorsed by the finding that animals even evolved to have a separate sense of taste for "fats" (51).

Therefore, the consensus is emerging that nutrient intake, genetic make-up and idiosyncratic metabolic disposition is needed to make a call on the "goodness" or "badness" of free-lipids and lipid-laden cells in a case-wise manner. More evaluation on how various cells, biomolecules and reactive species interact/react with adipocytes is necessary for us to understand the latter's role in pathology and physiological cascades. This could perhaps enable an adipose-tissue directed pharmacotherapy also (52).

Addressing the problematic ROS-lipid (adipocyte) interaction dynamics

There is a plethora of data supporting the malevolent role of ROS in several diseases. However, several other research groups have published data which support beneficial roles of ROS in those same diseases.

From the broad perceptions that ROS causes deterioration of lipids and lipid membranes, we now have specific information on how ROS react with lipid targets. These reactions most often lead to lipid peroxidations (9) which generates products like RCCs (reactive carbonyl compounds), alkanes and ketones. In turn, these products form cross-links and adducts with cellular constituents to form end products referred to as advanced lipid peroxidation end products (ALEs) (8). The RCCs can be further classified under three major groups (53):

1. the α,β-unsaturated aldehydes like 4-hydroxy-2-nonenal (4-HNE) and acrolein
2. di-aldehydes like malondialdehyde and glyoxal
3. cheto-aldehyde (methylglyoxal) and isoketals (levuglandins)

Some of these molecular species (as exemplified in 1 & 2 above) are implicated in artherosclerosis, chronic renal failure (54-58), neurodegenerative diseases like Alzheimers, Parkinsons, etc., (59-60), carcinogenesis (61-63) and chronic inflammatory diseases (64, 65, 66). All these diseases could potentially involve adverse ROS-lipid interactions. While species such as 4-HNE are implicated in disease and cell death, it should also be noted that evidence is also available where 4-HNE promotes cell survival and signaling in gene expression, antioxidant capacity, adaptive response and effect anti-cancer properties (67-71). It was argued therein that the outcomes depend on cellular metabolic circumstances and cell type, etc (72). Therefore, it can be seen that the interaction of ROS with adipocytes (and lipids therein) can also lead to positive effects.

Similarly, in some research studies, ROS are assigned a villainous role in the development of atherosclerosis or plaque formation in arteries (a condensed deposition of free lipids, calcium and miscellaneous substances that blocks the free flow of blood) (73-74). Nevertheless, in another study, Nox4, a ROS generating enzyme is implicated in anti-atherosclerotic function (75).

The same is the case for adipocytes. Some research groups provide evidence where adipocytes induce oxidative stress through release of ROS and ROS-inducing factors (76-79) and other groups give evidence of beneficial role of adipocytes in nutrient homeostasis, energy balance and release of endocrine signaling molecules and protein factors (18, 49, 50).

Though the distribution and dynamics of ROS have been studied in lipid bilayers (80), the real-time detection and quantification of various ROS (and their metabolic intermediates) is a major experimental challenge, as these species have highly dynamic and interactive equilibriums with their surrounding entities. Various labs use different probes with different settings. So, this could also lead to a major reason of disagreement be-

tween various research groups, and this predicament stands to pose significant problems in the times to come. Moving on, if we look at the conflicting research data presented, it is evident that ROS is benevolent when present in mild to moderate levels in specific loci and malevolent when present in high concentrations or in undesired loci. In this light, murburn concept, a new mechanistic paradigm, addresses the catalysis mediated by *in situ* generated ROS and diffusible reactive species (34-41, 43). This stochastic paradigm can give both selective/ordered reactions, and can also be subjected to fair amounts of uncertainties/chaos by the presence of trace amounts of additives. Therefore, the amphipathic lipids and their derivatives can give concentration-dependent and contrasting outcomes between systems and experimental setups. We have demonstrated some of the complex effects, underpinning mechanistic principles and constitutive controls (32, 35-36,43). In the murburn perspective, besides the classical affinity-based interactions, other facets like dynamic partitioning, relative concentrations, reaction microenvironments' dielectrics, availability of protons, spin state of reacting species, etc. are slated to be key governing constitutive principles that determine reaction outcomes (kinetics, stoichiometry, etc.). While it is clear how high amount of ROS can lead to deleterious effects, the tissue/loci-specific role change of ROS needs special attention, in the light of the contrasting findings and the possible explanations that murburn concept can afford. It has been proposed that timing, source and tissue specific effects of ROS would be beneficial in understanding the discrepancy of evidence presented in basic research and clinical studies (81). The tissue that is most evidently associated with ROS production and interaction is adipose tissue. Hence it is most important to keep *murburn concept* in perspective and examine the action of ROS within and outside adipose tissue to understand how ROS can have both deleterious and beneficial effects with respect to its location.

There is also emerging evidence that cellular liquid phases have membrane-less aggregates that compartmentalize molecules based on their chemical and physical properties. These structural aggregations also serve several functional attributes of the cell (82-85). We project that ROS-lipid interactive dynamics could affect such aggregations, thereby affecting normal or pathological physiology. For now, it is evident that the earlier viewpoints need to be revamped to accommodate the diverse set of experimental observations available. Clearly, not just in peroxisomes and phagocytes, ROS has an obligatory physiological role in mitochondria and the membranous cytoplasmic/sarcoplasmic reticulum. Therefore, ROS can no more be seen as a wasteful or toxic byproduct. Figure 3 captures the essential shift in perspective in this regard. As seen, lipids and lipid-laden tissues serve the function of retaining integrity, affording a platform for ROS reactions, and enable conservation of heat

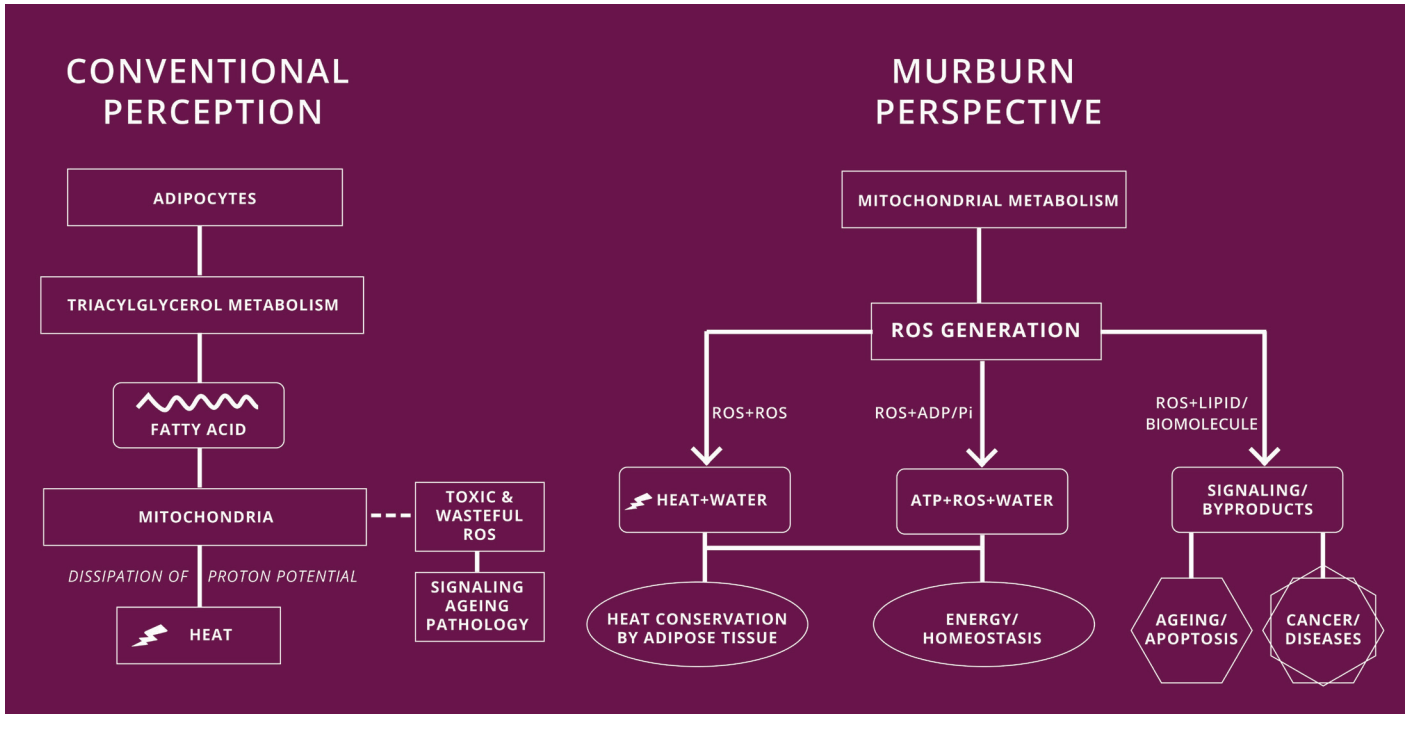

Figure 3. The interaction paradigms of ROS and lipids/adipocytes

by virtue of low heat capacity of oils (in comparison to water). It must be remembered that heat energy is one of the unsung heroes of metabolism- kinetics depend crucially on temperature! With these fundamental outlooks, reoriented research agenda can generate better understanding.

Conclusion

To recapitulate- it is true that DROS are implicated in oxidative stress, several diseases and pathophysiological states. It is also true that enzymes like catalase, peroxidase, SOD, etc. deplete DROS and some antioxidants alleviate harmful effects of DROS. However, in physiology, any entity has its utility with respect to the context of location, time and quantity. Our works have clearly shown that the aqueous phase redox enzymes (cited above) cannot compete for the small amounts of DROS dynamically generated at the phospholipid interface. Further, the thermodynamic, kinetic and reaction chemistry logic strongly endorse the utility of DROS in several metabolic and physiological processes. Just because a cut-injury in the kitchen most probably results from knife-abuse, one does not infer that knives do not have any positive role in the kitchen! Also, one may find gloves and cutting-boards to hold and handle knives in the kitchen. Their presence too does not imply that knives have no constructive culinary roles! (In this analogy- knives, gloves and cutting boards are equivalent to DROS, redox enzymes and antioxidants respectively.)

In the light of a plethora of emerging experimental evidences and theoretical insights, we should also acknowledge DROS' roles as catalysts and protagonists in cellular metabolism, homeostasis, energetics, and signaling.

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Conflict of interests

There are no conflicts of interests to disclose in the context.

Author contributions

Both VDJ and KMM wrote the paper. KMM conceived the ideas and VDJ made the figures.

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